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EFFECT OF POTASSIUM DEFICIENCY AND OF POTASSIUM DERIVED FROM DIFFERENT SOURCES ON THE COMPOSITION OF THE JUICE OF VALENCIA ORANGES[1]

BY WALLACE R. ROY

Associate biochemist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture[2]

INTRODUCTION

The work reported herein was undertaken for the purpose of determining the progressive changes in some of the biochemical characteristics of the juice of orange (*Citrus sinensis* (L.) Osbeck) when the fruit was grown under controlled fertilization, the only variable nutritional factor being the amount and source of potash. This study constitutes one phase of an experimental project of the Bureau of Plant Industry, Soils, and Agricultural Engineering which has been in progress for 5 years at Orlando, Fla. The results of certain other phases of the experiment have already been reported (*6*).[3]

The specific factors considered in the present work concern the progressive changes in the composition of the juice, during the final 6 months of development of the fruit, with respect to sugars, ascorbic acid, buffer capacity, citric acid, ash and its alkalinity, and potassium, when the trees were fertilized with potash from different sources and applied at different levels. All other fertilizer ingredients were applied at a constant level. The application levels ranged as high as twice the customary levels.

Many Florida citrus growers hesitate to use potash from chloride sources in their fertilizer mixes, mainly because of the deleterious effect that fertilizers high in chlorine are known to have on such crops as tobacco and potatoes, although evidence of any harmful effects from the moderate use of chloride salts on citrus is lacking. Hume (*28*) stated:

> Of all the materials [sources of potash] the sulfates are safest and best. There is a general impression that muriate of potash is not a good substance to use on orange trees, and while its deleterious effects have never been elucidated, the impression is probably well grounded.

Since the muriate salts of potash are less expensive per unit of potash than the sulfate or the sulfate of potash-magnesia, it is highly desirable from an economic point of view to ascertain whether there is any basis for the prejudice against their use.

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[2] The author wishes to express his appreciation to the late R. A. Gortner, Division of Agricultural Biochemistry, University of Minnesota, for his suggestions in outlining the work described herein; to J. J. Skinner and G. M. Bahrt, Bureau of Plant Industry, Soils, and Agricultural Engineering, for assistance in planning the experiment; and to Paul L. Harding, J. M. Bellows, Jr., and F. E. Gardner, Bureau of Plant Industry, Soils, and Agricultural Engineering, for helpful suggestions in the preparation of the manuscript.

[3] Italic numbers in parentheses refer to Literature Cited, p. 167.

The nutritional value of orange juice, as pointed out by Jones (*29*), is probably due to three main properties, each measurable by chemical means. These are the vitamin C content, the alkalizing value, and the caloric value, the last depending mainly on the sugar content. The principal nutritional value of oranges lies in their vitamin C content. The alkalizing value is also considered to be important (*10*), but less stress seems to have been placed on the actual energy value of the fruit.

In recent years both growers and consumers have become more conscious of quality in citrus fruit. This factor has been strongly emphasized at meetings of leaders of the citrus industry in Florida, in which the need for a "medicinal" grade of citrus fruit has been discussed; i. e., a fruit with an exceptionally high content of ascorbic acid, ash, and sugar and one that can be specifically recommended for use by invalids and children. It is important, therefore, to know how far and in what manner potash contributes to the production of fruit possessing these characteristics.

REVIEW OF LITERATURE

Although the necessity of potassium for plant growth has long been recognized, its exact role in the development of plants is not known. As Miller (*33, p. 318*) has stated, " . . . its function is only surmised by noting the effects on the plant in its absence."

Much of the experimental work with potash has been carried on to determine its effect on yield of the crop. Such investigations on citrus fruits have shown that yields can be increased by the inclusion of potash in the fertilizer, but that the point of diminishing returns from potash applications varies, depending upon the soil type and its natural fertility with respect to this element (*6; 39; 44, pp. 44, 45; 45, p. 47*). This latter fact has probably influenced conclusions such as those reached by Young (*52*), who reported no effect of potash fertilization on the composition of the orange but stated that the soil used in his studies was naturally well supplied with potash.

Light soils, such as are found in Florida, lend themselves readily to fertilization experiments. In 1894, Webber (*50*), basing his fertilizer recommendations on observations of intelligent citrus growers, suggested that potash applications be decreased and sulfate of ammonia be used when it was desirable to sweeten the fruit and that potash application be increased if a more acid fruit were preferred.

Anderssen (*2*) stated that potassium does not influence the sugar content of oranges, but like Young he used a heavy soil. He correlated high potash fertilization with high juice acidity. Morris' (*35*) findings were similar under similar conditions. Benton and Stokes (*8*) reported that the total sugar content of orange juice was less in fruit from trees fertilized with large amounts of potash. Colby (*13*) found an inverse relation between potash fertilization and the concentration of sugar in orange juice.

The American Medical Association (*1, p. 365*) and Daniel and Munsell (*16*) listed oranges as among the richest sources of vitamin C. Roy and Bahrt (*42*) and Fudge and Fehmerling (*19*) showed that a relation exists between the vitamin C content of oranges and various soil fertility factors. Nelson (*36*) found that repeated spraying with arsenicals caused a decrease in the vitamin C content of oranges.

Kinman (*30*) reported that the flavor of oranges grown without potash was insipid. This statement could be interpreted to mean that the citric acid content was less than that of well-fertilized fruit of the same age. Hilgeman et al. (*27*) found that when potash was withheld for 3 years from grapefruit trees, the fruit juice contained less acid than that of fruit produced on well-fertilized plots.

McKaig and Hurst (*31*) found that application of potassium increased the pH values, the ash, and the potassium content of sugarcane, but Hartt (*24*) could establish no differences in pH or in titratable acid attributable to the element. She found that the amount of potassium in the ash of sugarcane was directly proportional to the amount of the element supplied in the nutrient solution. In her studies potassium was the only element that showed any strong tendency to migrate.

Haas (*20*) could find no evidence of any relation between the potassium in the ash of orange juice and that in the soil. The amount of potassium in the whole fruit, however, was increased by soil fertilization with potash.

Both the citric acid content (*14, 18, 22*) and the ascorbic acid content (*18, 21, 51*) of citrus fruit juices have been shown to decrease periodically during the maturing process. The sugar content, on the other hand, progressively increases. Collison (*14*) reported that the concentration of sucrose increases at a faster rate than does that of the reducing sugars. Harding et al. (*22*) noted the same seasonal trend, while Braverman and Carmi (*12, p. 152*) reported that sucrose increased but that the reducing sugars remained constant in the juice throughout the season. The last-named workers based their conclusions on analyses of Palestine oranges. Hilgeman and Smith (*26*) called attention to the fact that grapefruit, when mature, contain a maximum amount of sucrose.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

The experiment was performed on a block of uniform bearing Valencia orange trees set out in 1924. This block is located about 1 mile east of Narcoossee, Fla. The trees were budded on rough lemon rootstocks. The data reported in this paper were derived from fruit of the 1939–40 crop after the fertilizer applications had been continued for 5 to 5½ years.

The soil type was Norfolk fine sand, a soil widely planted to citrus throughout the State and adaptable to experimentation because of its permeability and low mineral and organic-matter content.[4] As the location was exposed to 55 or 60 inches of rainfall, there was a minimum of residue of soil nutrients from previous cultural treatments.

The soil reaction was fairly uniform throughout the grove, ranging from pH 4.5 to 5.5. A soil of this type containing little organic matter or colloidal material readily reflects by its pH values the presence of residual substances from previous fertilizer applications. Moreover the pH values of such soils can easily be altered in a rather short time. Although citrus trees grow in soils covering a wide range of pH values

[4] Peech (*37*) reported the following figures from analyses of 50 samples of Norfolk fine sand: Exchange capacity, 1.67 to 5.00 milliequivalents; organic matter, 0.93 to 3.10 percent.

and the opinions of growers differ widely as to which is best for the growth of citrus trees, a pH value of approximately 6.0 is usually considered the most desirable.

The fertilizers used were nearly neutral in reaction (residual effect), and there was little or no change in the pH of the soils in the experimental plots during the course of the experiment.

Valencia orange trees were selected for the experiment because this variety is the predominant late orange grown in the State. It might be mentioned that rough lemon rootstock is particularly adapted to well-drained light sandy soil.

Each experimental plot consisted of five trees in a row, with the exception of plots 27 and 29, which contained seven trees each. Each

O	O	O	O	O	O	O	O	O	O	O	O	O	O
O	5X	O	6X	O	7X	O	8X	O	9X	O	10X	O	11X	O
O	5X	O	6X	O	7X	O	8X	O	9X	O	10X	O	11X	O
O	5X	O	6X	O	7X	O	8X	O	9X	O	10X	O	11X	O
O	5X	O	6X	O	7X	O	8X	O	9X	O	10X	O	11X	O
O	5X	O	6X	O	7X	O	8X	O	9X	O	10X	O	11X	O
O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
O	12X	O	13X	O	14X	O	15X	O	16X	O	17X	O	18X	O
O	12X	O	13X	O	14X	O	15X	O	16X	O	17X	O	18X	O
O	12X	O	13X	O	14X	O	15X	O	16X	O	17X	O	18X	O
O	12X	O	13X	O	14X	O	15X	O	16X	O	17X	O	18X	O
O	12X	O	13X	O	14X	O	15X	O	16X	O	17X	O	18X	O
O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
O	19X	O	20X	O	21X	O	22X	O	23X	O	24X	O	25X	O
O	19X	O	20X	O	21X	O	22X	O	23X	O	24X	O	25X	O
O	19X	O	20X	O	21X	O	22X	O	23X	O	24X	O	25X	O
O	19X	O	20X	O	21X	O	22X	O	23X	O	24X	O	25X	O
O	19X	O	20X	O	21X	O	22X	O	23X	O	24X	O	25X	O
O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
O	27X	27X	27X	27X	27X	27X	27X	O	26X	26X	26X	26X	26X	O
O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
O	29X	29X	29X	29X	29X	29X	29X	O	28X	28X	28X	28X	28X	O
O	O	O	O	O	O	O	O	O	O	O	O	O	O	O

FIGURE 1.—Plan of experiment on Valencia oranges grown on rough lemon rootstocks, Narcoossee, Fla., 1935–40. X, Trees in experimental plots. O, Trees in buffer rows. The figures are plot numbers.

plot was separated from adjacent plots by a row of buffer trees (fig. 1). By thus isolating each treatment the effects of adjoining fertilizer applications were minimized. Each treatment was replicated three or four times at random.

FERTILIZER APPLICATIONS

The compositions and grades of the fertilizers used are shown in table 1. For the sake of brevity the treatments in which no potassium was used are termed the "no-potash" treatments; those which consisted of an annual application of 1.7 to 2.3 pounds [5] of potash per tree are called the "medium" muriate, sulfate, or sulfate of potash-magnesia treatments, while those in which 2.7 to 3.6 pounds of potash

[5] In the fall of 1934, a single application of 10 pounds per tree was made. During 1935 three applications of 10 pounds each per tree were made. The annual application per tree was increased by 2 pounds each succeeding year thereafter, so that in 1939, each tree received a total of 38 pounds of fertilizer.

per tree was applied annually are referred to as the "high" muriate, sulfate, or sulfate of potash-magnesia treatments.

TABLE 1.—*Composition and grades of fertilizers used in the experiment with Valencia oranges grown on rough lemon rootstocks, Narcoossee, Fla., 1935–40*

Treatment	Potash applied per tree per year	Fertilizer [1]		Plot numbers
		When applied	Grade (N-P_2O_5-K_2O)	
	Pounds			
No potash	None	Spring	4-8-0	5, 12, 22, 26
		Summer	3-8-0	
		Fall	3-8-0	
Medium sulfate of potash	1.7 to 2.3	Spring	4-8-4	6, 16, 21, 24
		Summer	3-8-5	
		Fall	3-8-8	
Medium muriate of potash	1.7 to 2.3	Spring	4-8-4	9, 13, 25
		Summer	3-8-5	
		Fall	3-8-8	
Medium sulfate of potash-magnesia	1.7 to 2.3	Spring	4-8-4	7, 17, 19, 23
		Summer	3-8-5	
		Fall	3-8-8	
High sulfate of potash	2.7 to 3.6	Spring	4-8-4	10, 14, 20
		Summer	3-8-10	
		Fall	3-8-13	
High muriate of potash	2.7 to 3.6	Spring	4-8-4	8, 18, 28
		Summer	3-8-10	
		Fall	3-8-13	
High sulfate of potash-magnesia	2.7 to 3.6	Spring	4-8-4	11, 15, 29
		Summer	3-8-10	
		Fall	3-8-13	

[1] Potash source indicated in description of treatment; potash derived from 50 percent muriate of potash, 48 percent sulfate of potash, and 26 percent sulfate of potash-magnesia; phosphoric acid from superphosphate and steamed bonemeal; nitrogen, one-half from sodium nitrate and ammonium sulfate and one-half from steamed bonemeal, cottonseed meal, and tankage.

Fertilizer was applied three times a year; the spring application was made in March, the summer application in July, and the fall application in November or December. The mix was broadcast by hand evenly over the entire area defined by the treatment, from tree trunk to tree trunk, to insure an even distribution over the entire root-system area.

All fertilizers were formulated and mixed by or under the supervision of employees of the United States Department of Agriculture. The materials used were all of the market grade customarily applied.

Insect control was conducted by the owner of the grove, and all spray and dust applications were made uniformly throughout the grove. A volunteer cover crop was allowed to grow and was disked in the fall. All other cultural practices, such as cultivation and pruning, were carried on by the owner of the grove.

SAMPLING

The first samples were taken from the trees on September 16, 1939, when the fruit was of sufficient size to yield adequate amounts of juice for analysis. Thereafter samples were obtained at intervals of approximately 3 weeks[6] throughout the growing season until March 8, 1940, when the grove was picked commercially.

[6] An exception to this schedule occurred unavoidably between October 7 and November 4, a lapse of 4 weeks.

Forty oranges were picked from each plot, the procedure being to clip the fruit from the periphery of the tree at a height of about 5 feet. Appleman and Richards (*4*) showed statistically that samples of oranges consisting of less than 20 to 30 fruits constitute inadequate representation of the supply when conclusions are to be derived from data obtained from such samples. Harding et al. (*22*) demonstrated the adequacy of orange samples of 25 or more fruits as being fully representative of the supply.

No attempt was made to select fruit for size or color. The only precaution observed was to avoid taking late-bloom fruit.

The fruit from each plot was placed in labeled bags and carried to the laboratory where it was carefully washed and surface-dried. Each fruit was cut in half, and the juice was lightly expressed by hand squeezing, care being taken to keep the sample as free from rind oil as possible. The juice was then filtered through two thicknesses of clean cheesecloth and thoroughly mixed.

Samples were taken immediately for titratable acidity, ascorbic acid, and sugar determination. About 250 ml. of the remaining juice was stored in stoppered Erlenmeyer flasks at 0° C. until further samples were needed for other determinations. All determinations were completed in the 3-week intervals between samplings. The sequence of making the analyses for the various constituents was based upon the susceptibility to change of the constituents, that is, the most labile substances such as ascorbic acid and sugars were determined at once, whereas the citric acid, ash and its alkalinity, and buffer capacity were determined later in the intersampling period.

SUGARS

Reducing sugars were determined by the application of a modification of the Scales method (*43*) after first clarifying a weighed sample of the orange juice with neutral lead acetate. Total sugars were determined by the use of the same method on a clarified sample of the juice which had been inverted in the presence of 0.6 N hydrochloric acid at 25° C. for 12 hours. Nonreducing sugars were calculated from the difference between total and reducing sugars by utilizing appropriate conversion factors. Reducing sugars were calculated as glucose and nonreducing as sucrose.

ASCORBIC ACID

Ascorbic acid was determined by titrating a 5-ml. sample of the juice in 10 ml. of 8 percent acetic acid with an aqueous solution of sodium 2,6-dichlorobenzenoneindophenol, which had been standardized against a solution of pure ascorbic acid. The end point observed was a faint pink color, which persisted for 30 seconds. The method was essentially that described by Bessey and King (*9*). All solutions used were prepared immediately before use, as it has been found that ascorbic acid solutions slowly deteriorate on aging, and the redox dye yields a rather indefinite muddy end point after aging for 3 or 4 days. The powdered dye was thoroughly washed with anhydrous ethyl ether before being dissolved in water, and its strength was adjusted so that a titration of 5 to 10 ml. was obtained by the use of a 5-ml. sample of orange juice. As is customary, the ascorbic acid content of the juice is reported as milligrams per milliliter of juice.

CITRIC ACID

Citric acid was determined by precipitation from a sample of the juice with lead acetate and isolating the salt by centrifuging and washing. Lead was precipitated with hydrogen sulfide and removed by filtration. The citrate was oxidized and brominated to form pentabromacetone, which was collected on a tared Gooch crucible, dried for 24 hours over anhydrous sulfuric acid in a vacuum desiccator, and weighed. The percentage of citric acid in the original juice was calculated from these data. The method is that described by the Association of Official Agricultural Chemists (*5*).

The titratable acidity (*6*) was determined by titrating a sample of the fresh juice with standard sodium hydroxide solution; phenolphthalein was used as the indicator. The acidity was calculated as percentage of citric acid.

BUFFER CURVES AND INDICES

Buffer curves were obtained by the following procedure. The pH of a 50-ml. sample of the juice was determined by means of the quinhydrone electrode. Increments of 3 ml. of 0.5 N hydrochloric acid were added. The pH of the solution was determined after each addition. This procedure was repeated until the pH of the solution was 1.0 or less. A new 50-ml. portion of the juice was then treated with increments of 3 ml. of 0.5 N sodium hydroxide, the pH being determined after each addition of base until a pH of 9.0 was attained.

From data so obtained curves were drawn with pH values as the abscissas and gram equivalents of acid or alkali as ordinates.

The same data were employed to plot the buffer indices as defined by Van Slyke (*49*), the graphs representing the slopes of the pH-titration curves $\frac{(dB)}{(dpH)}$ as ordinates and pH values as abscissas.

ASH AND ITS ALKALINITY

Ash was determined by weighing a 5-gm. sample of juice in a small porcelain crucible, drying it in an oven at 105° C. for 24 hours, and then ashing to constant weight at 400° to 500° in an electric muffle furnace.

The ash was dissolved in an excess of 0.1 N hydrochloric acid, gently heated to insure complete solution, cooled, and titrated with 0.1 N sodium hydroxide; methyl orange was used as the indicator. The alkalinity of the ash was reported as the number of milliliters of 0.1 N hydrochloric acid required to neutralize the ash from a 100-gm. sample (*5*).

POTASSIUM

Potassium was determined in the sample used for ash and alkalinity of the ash. It was determined as the chloroplatinate, as described by the Association of Official Agricultural Chemists (*5*).

STATISTICAL TREATMENT

The data obtained were analyzed statistically according to methods outlined by Fisher (*17*) and Snedecor (*48*). Analysis of variance was applied to determine the significance of differences both between seasonal means of treatments and between treatments for the last sampling on March 8, 1940, when the fruit was harvested commercially.

Data from the last sampling were the only single sampling-date figures subjected to statistical analysis because, being from the harvest sample, they represented analyses of juice from fruit as actually bought by the consumer. Differences between means were considered significant when they attained or exceeded the 5-percent level, and highly significant at or above the 1-percent level.

Correlations were established by applying the method of least squares for regression curves. Correlation coefficients were determined by application of formulas given by the above-mentioned texts (*17*, *48*).

RESULTS

SUGARS

Data showing the influence of potash on the reducing sugar, nonreducing sugar, and total sugar content of fruit from the seven treatments during the period of study are presented in table 2. The data are shown graphically in figure 2.

TABLE 2.—*Sugar content of Valencia orange juice on various dates during the maturation period, 1939–40*

[Means of plot replicates]

Sugar determined and potassium treatment	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
Reducing sugars (as glucose):										
No potash	3.89	4.45	4.71	5.13	5.68	5.98	5.64	5.96	6.72	5.35
Medium sulfate of potash	3.14	3.61	3.72	4.01	4.61	4.70	4.73	5.09	5.61	4.36
Medium muriate of potash	3.04	3.40	3.55	3.78	4.43	4.45	4.40	4.80	5.27	4.12
Medium sulfate of potash-magnesia	3.07	3.55	3.71	3.87	4.51	4.64	4.69	4.89	5.42	4.26
High sulfate of potash	2.82	3.43	3.55	3.72	4.27	4.34	4.44	4.81	5.42	4.09
High muriate of potash	3.08	3.59	3.64	3.85	4.54	4.53	4.44	4.77	5.28	4.19
High sulfate of potash-magnesia	3.04	3.69	3.73	4.02	4.68	4.70	4.76	5.11	5.56	4.37
Difference required for significance:										
5-percent level									.500	.110
1-percent level									.759	.154
Nonreducing sugars (as sucrose):										
No potash	1.37	1.83	2.28	3.61	4.28	4.48	4.47	4.32	4.50	3.46
Medium sulfate of potash	1.31	1.83	2.46	3.75	4.55	4.69	4.84	4.62	4.67	3.64
Medium muriate of potash	1.34	1.76	2.25	3.74	4.38	4.51	4.86	4.67	4.79	3.59
Medium sulfate of potash-magnesia	1.28	1.66	2.26	3.68	4.46	4.72	4.74	4.80	4.97	3.62
High sulfate of potash	1.12	1.81	2.32	3.57	4.49	4.56	4.88	4.74	5.04	3.61
High muriate of potash	1.48	1.81	2.49	3.71	4.49	4.65	4.94	4.51	4.73	3.64
High sulfate of potash-magnesia	1.41	1.83	2.43	3.81	4.52	4.66	5.25	4.69	5.05	3.74
Difference required for significance:										
5-percent level									(1)	.147
1-percent level									(1)	.204
Total sugars:										
No potash	5.26	6.23	6.99	8.74	9.96	10.46	10.11	10.27	11.22	8.80
Medium sulfate of potash	4.45	5.43	6.18	7.92	9.16	9.39	9.57	9.71	10.28	8.01
Medium muriate of potash	4.33	5.15	5.80	7.52	8.81	8.98	9.25	9.47	10.06	7.71
Medium sulfate of potash-magnesia	4.35	5.21	5.97	7.55	8.97	9.36	9.43	9.69	10.38	7.88
High sulfate of potash	3.95	5.24	5.87	7.28	8.75	8.91	9.38	9.55	10.45	7.71
High muriate of potash	4.56	5.40	6.13	7.56	8.94	9.16	9.38	9.29	10.01	7.83
High sulfate of potash-magnesia	4.45	5.51	6.16	7.84	9.20	9.36	10.01	9.81	10.61	8.11
Difference required for significance:										
5-percent level									(1)	.180
1-percent level									(1)	.251

[1] Differences not significant.

In general all the sugars studied showed gradual but regular increases in orange juice from all treatments as the fruit ripened. The mean concentration of reducing sugar was found to be significantly higher in juice from the no-potash plots than in that from any of the potash-treated plots, when the season as a whole was considered.

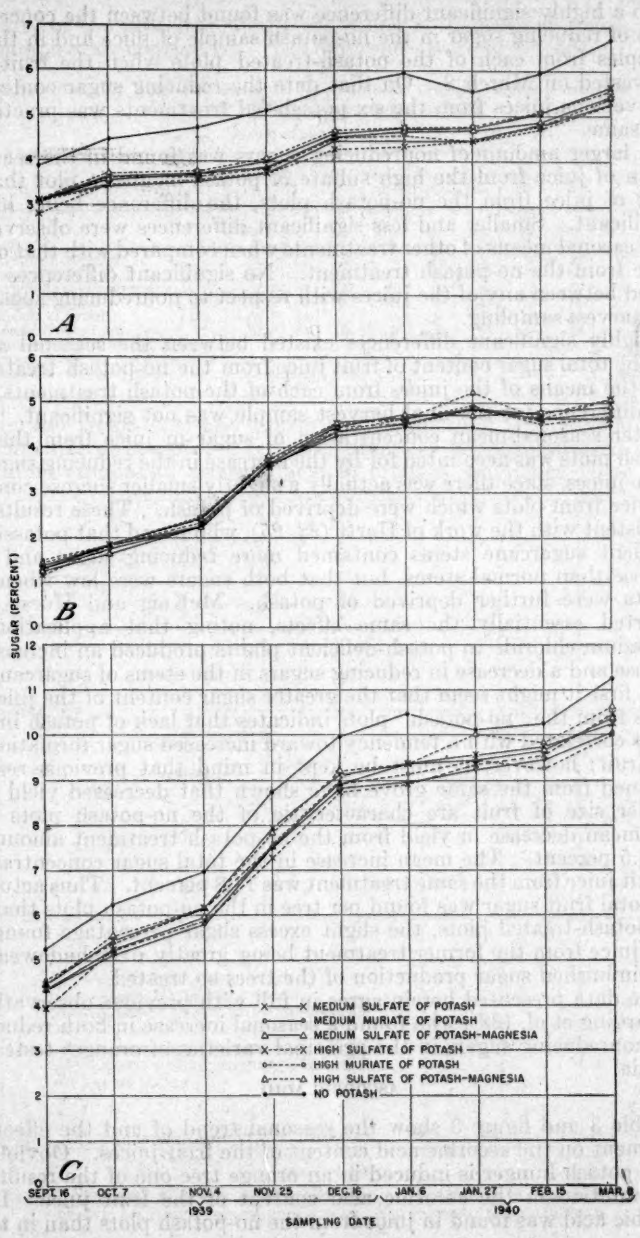

FIGURE 2.—Seasonal changes in the sugar content of Valencia oranges grown on rough lemon rootstocks, Narcoossee, Fla., 1939–40: *A*, Reducing sugars (glucose); *B*, nonreducing sugars (sucrose); *C*, total sugars.

Also a highly significant difference was found between the concentration of reducing sugar in the no-potash sample of juice and in that of samples from each of the potash-treated plots when the fruit was harvested on March 8. On that date the reducing sugar content of the various juices from the six potash-fed treatments was practically the same.

A larger amount of nonreducing sugars was found in the seasonal mean of juice from the high sulfate of potash-magnesia plot than in that of juice from the no-potash plots, the difference being highly significant. Smaller and less significant differences were observed in the seasonal means of other treatments when compared with that of the juice from the no-potash treatment. No significant differences were noted between any of the juices with respect to nonreducing sugars at the harvest sampling.

Highly significant differences existed between the seasonal mean for the total sugar content of fruit juice from the no-potash treatment and the means of the juices from each of the potash treatments, but the difference for the final harvest sample was not significant. The greater seasonal-mean concentration of sugar in juice from the no-potash plots was accounted for by the increase in the reducing sugar in those juices, since there was actually a slightly smaller sucrose content in juice from plots which were deprived of potash. These results are consistent with the work of Hartt (*23*, *25*), who found that potassium-deficient sugarcane stems contained more reducing sugar and less sucrose than normal stems, but that both sugars were low when the plants were further deprived of potash. McKaig and Hurst (*31*) reported essentially the same effects, noting that application of potassium chloride to potash-deficient plants produced an increase in sucrose and a decrease in reducing sugars in the stems of sugarcane.

At first it might seem that the greater sugar content of the juice of fruits from the "no-potash" plots indicates that lack of potash in the soil is correlated with a tendency toward increased sugar formation in the fruit; however, it must be kept in mind that previous results obtained from the same grove have shown that decreased yield and smaller size of fruit are characteristic of the no-potash plots (*6*). The mean decrease in yield from the no-potash treatment amounted to 18.5 percent. The mean increase in the total sugar concentration of fruit juice from the same treatment was 11.8 percent. Thus actually less total fruit sugar was found per tree in the no-potash plots than in the potash-treated plots, the slight excess sugar percentage found in fruit juice from the former treatment being greatly overshadowed by the diminished sugar production of the trees so treated.

The data presented herein agree in full with previous observations of Harding et al. (*22*), who found a seasonal increase in both reducing and nonreducing sugars in the principal varieties of oranges tested in Florida.

ASCORBIC ACID

Table 3 and figure 3 show the seasonal trend of and the effect of treatment on the ascorbic acid content of the fruit juices. Obviously when potash hunger is induced in an orange tree one of the results is a diminution in the ascorbic acid content of the fruit juice. Less ascorbic acid was found in juice from the no-potash plots than in that

from any of the potash-treated plots, when both seasonal means and final sample were considered. The differences were significant.

TABLE 3.—*Ascorbic acid per milliliter of Valencia orange juice on various dates during the maturation period, 1939–40*

[Means of plot replicates]

Treatment	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
No potash	0.55	0.53	0.51	0.51	0.44	0.43	0.46	0.45	0.45	0.48
Medium sulfate of potash	.61	.60	.58	.58	.52	.51	.56	.52	.51	.55
Medium muriate of potash	.60	.59	.56	.57	.51	.49	.53	.50	.50	.54
Medium sulfate of potash-magnesia	.61	.58	.57	.56	.50	.49	.53	.50	.49	.54
High sulfate of potash	.61	.60	.58	.59	.51	.49	.56	.53	.52	.55
High muriate of potash	.60	.59	.57	.58	.51	.49	.54	.51	.48	.54
High sulfate of potash-magnesia	.61	.60	.58	.58	.51	.51	.56	.52	.49	.55
Differences required for significance:										
5-percent level	------	------	------	------	------	------	------	------	.027	.009
1-percent level	------	------	------	------	------	------	------	------	.041	.013

Roy and Bahrt (*42*) and Fudge and Fehmerling (*19*) have shown experimentally that proper fertilizer practices are accompanied by a maximum amount of ascorbic acid in oranges, but when there is a

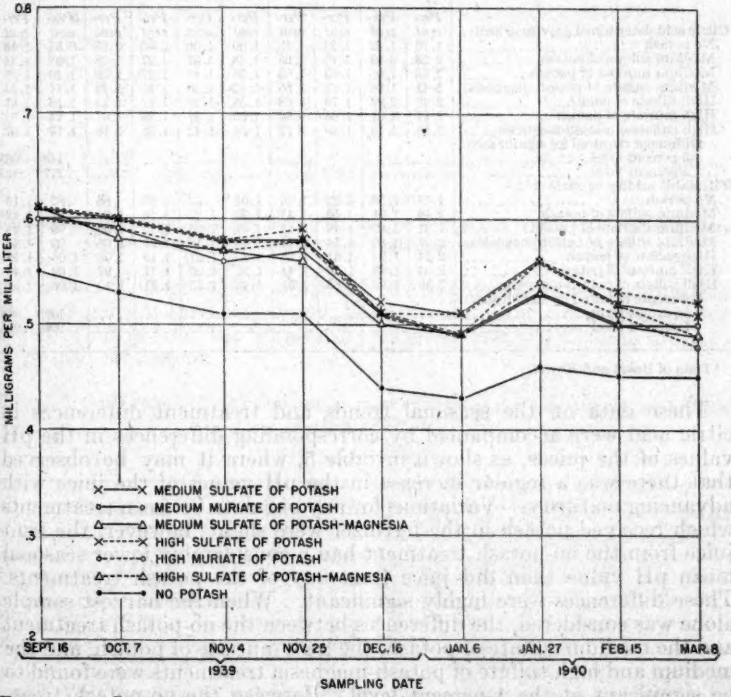

FIGURE 3.—Seasonal changes in the ascorbic acid content of Valencia oranges grown on rough lemon rootstocks, Narcoossee, Fla., 1939–40.

deficiency of any one of several elements the ascorbic acid content of the juice is lowered.

CITRIC ACID, pH, AND BUFFERS

When routine analyses of juice from mature oranges are made, the determination of citric acid consists of a titration of a sample of the juice with standardized alkali and the calculation of the acidity as citric acid. In the present experiments, however, analyses of actual citric acid were also made (table 4, fig. 4). The customary seasonal decrease in the citric acid content was apparent. Highly significantly smaller amounts of citric acid were found in the fruit juice from the no-potash plots than in that from any of the potash plots when both seasonal means and the sampling of March 8 were considered. The close agreement between the actual citric acid determined as such and the citric acid content of the juice calculated from the titratable acidity is worthy of note.

TABLE 4.—*Citric acid in Valencia orange juice on various dates during the maturation period, 1939–40*

[Means of plot replicates]

Method of determining citric acid and treatment	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
Citric acid determined gravimetrically:										
No potash	1.76	1.42	1.32	1.19	1.09	1.00	0.95	0.85	0.84	1.16
Medium sulfate of potash	2.23	2.03	1.70	1.63	1.48	1.37	1.27	1.08	1.08	1.54
Medium muriate of potash	2.33	1.96	1.63	1.65	1.58	1.42	1.27	1.09	1.10	1.55
Medium sulfate of potash-magnesia	2.41	1.98	1.61	1.56	1.42	1.30	1.31	1.12	1.11	1.54
High sulfate of potash	2.48	2.04	1.79	1.68	1.58	1.36	1.27	1.15	1.16	1.61
High muriate of potash	2.14	2.01	1.66	1.66	1.55	1.37	1.28	1.07	1.14	1.54
High sulfate of potash-magnesia	2.56	2.13	1.89	1.72	1.54	1.42	1.38	1.16	1.19	1.67
Difference required for significance:										
5-percent level									.143	.088
1-percent level									.217	.123
Titratable acidity as citric acid:[1]										
No potash	1.78	1.50	1.22	1.16	1.06	.97	.97	.86	.82	1.15
Medium sulfate of potash	2.16	1.91	1.50	1.47	1.33	1.22	1.16	1.01	.98	1.42
Medium muriate of potash	2.31	1.89	1.49	1.43	1.39	1.26	1.15	1.00	.99	1.43
Medium sulfate of potash-magnesia	2.27	1.89	1.54	1.44	1.36	1.18	1.21	1.05	.99	1.44
High sulfate of potash	2.34	1.94	1.61	1.55	1.40	1.21	1.19	1.03	1.06	1.48
High muriate of potash	2.05	1.89	1.49	1.43	1.35	1.26	1.17	.97	1.02	1.40
High sulfate of potash-magnesia	2.39	1.95	1.65	1.55	1.43	1.35	1.21	1.10	1.06	1.52
Difference required for significance:										
5-percent level									.109	.068
1-percent level									.166	.095

[1] Data of Bahrt and Roy (6).

These data on the seasonal trends and treatment differences in citric acid were accompanied by corresponding differences in the pH values of the juices, as shown in table 5, where it may be observed that there was a regular increase in the pH values of the juice with advancing maturity. Variations for any one date between treatments which received potash in the fertilizer were slight; however, the fruit juice from the no-potash treatment had a considerably lower seasonal mean pH value than did juice from any of the potash treatments. These differences were highly significant. When the harvest sample alone was considered, the differences between the no-potash treatment and the medium sulfate of potash, the high muriate of potash, and the medium and high sulfate of potash-magnesia treatments were found to be significant at the 1-percent level. Between the no-potash treat-

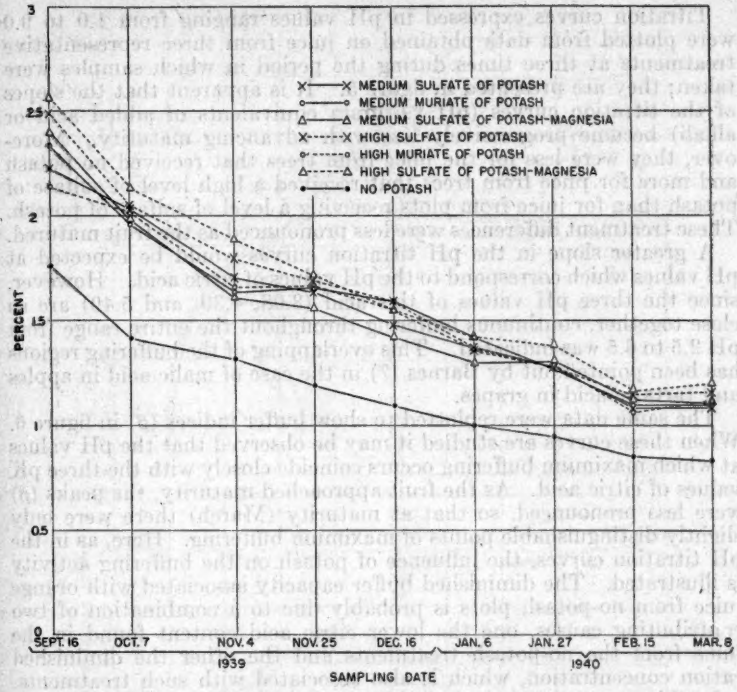

FIGURE 4.—Seasonal changes in the citric acid content of Valencia oranges grown on rough lemon rootstocks, Narcoossee, Fla., 1939–40.

ments and the high sulfate of potash treatments a significant difference at the 5-percent level was noted, but no real differences were observed in pH of juices from the no-potash treatment and the medium muriate of potash treatment.

TABLE 5.—*pH values of Valencia orange juice on various dates during the maturation period, 1939–40*

[Means of plot replicates]

Treatment	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
No potash	2.69	2.74	2.84	2.95	3.00	3.10	3.21	3.28	3.28	3.01
Medium sulfate of potash	2.79	2.81	2.97	3.06	3.12	3.18	3.29	3.31	3.41	3.10
Medium muriate of potash	2.78	2.83	2.98	3.05	3.13	3.19	3.29	3.39	3.33	3.11
Medium sulfate of potash-magnesia	2.75	2.82	2.95	3.05	3.12	3.20	3.28	3.35	3.45	3.11
High sulfate of potash	2.79	2.83	2.96	3.05	3.13	3.24	3.30	3.31	3.36	3.11
High muriate of potash	2.81	2.81	2.99	3.08	3.12	3.20	3.33	3.41	3.37	3.12
High sulfate of potash-magnesia	2.77	2.82	2.93	3.03	3.13	3.17	3.27	3.33	3.40	3.09
Difference required for significance:										
5-percent level									.056	.029
1-percent level									.085	.041

Titration curves expressed in pH values ranging from 1.0 to 9.0 were plotted from data obtained on juice from three representative treatments at three times during the period in which samples were taken; they are presented in figure 5. It is apparent that the slopes of the titration curves (pH vs. gram equivalents of added acid or alkali) became progressively less with advancing maturity. Moreover, they were less for the juice from trees that received no potash and more for juice from trees that received a high level of sulfate of potash than for juice from plots receiving a level of sulfate of potash. These treatment differences were less pronounced as the fruit matured.

A greater slope in the pH titration curves would be expected at pH values which correspond to the pH values of citric acid. However, since the three pH values of this acid (3.08, 4.39, and 5.49) are so close together, continuous buffering throughout the entire range from pH 2.5 to 6.5 was indicated. This overlapping of the buffering regions has been pointed out by Barnes (*7*) in the case of malic acid in apples and tartaric acid in grapes.

The same data were replotted to show buffer indices (β) in figure 6. When these curves are studied it may be observed that the pH values at which maximum buffering occurs coincide closely with the three pK values of citric acid. As the fruit approached maturity, the peaks (β) were less pronounced, so that at maturity (March) there were only slightly distinguishable points of maximum buffering. Here, as in the pH titration curves, the influence of potash on the buffering activity is illustrated. The diminished buffer capacity associated with orange juice from no-potash plots is probably due to a combination of two contributing causes, one the lower citric acid content found in the juice from the no-potash treatments and the other the diminished cation concentration, which is also associated with such treatments.

In this connection it is desirable to point out an apparent, though not a real, disagreement with data presented by Harding et al. (*22*). These authors determined the buffer values of seasonal samples of orange juice and reported that the values increased with maturity, whereas the results reported herein indicate a decrease with maturity. However, they titrated only to the acid side of the original pH of the juice and to pH 1.5, which corresponds to the acidity of normal gastric juice. Their interpretation of buffer value was adopted from the definition suggested by Moore (*34*), which is "that amount of tenth normal HCl required to change one gram of food from its initial pH to a pH of 1.5." Since the initial pH of the juice increased with advancing maturity, the actual pH range through which the juice must pass to attain a pH of 1.5 is greater as maturity progresses, with the result that an increase occurs in the buffer value according to Moore's definition. However, when the buffer indices of orange juice are considered over the range of pH 1.0 to 9.0, it is seen that these values decrease with maturity.

The close agreement between the values obtained for citric acid by titration and for actual citric acid determined gravimetrically indicates that citric is the predominant organic acid in orange juice. This conclusion is further strengthened by the evidence that pH values at points of maximum buffering coincide closely with the pK values of citric acid. Braverman (*11*) reported the presence of malic, tartaric, and succinic acids in Palestine oranges in addition to citric acid, but

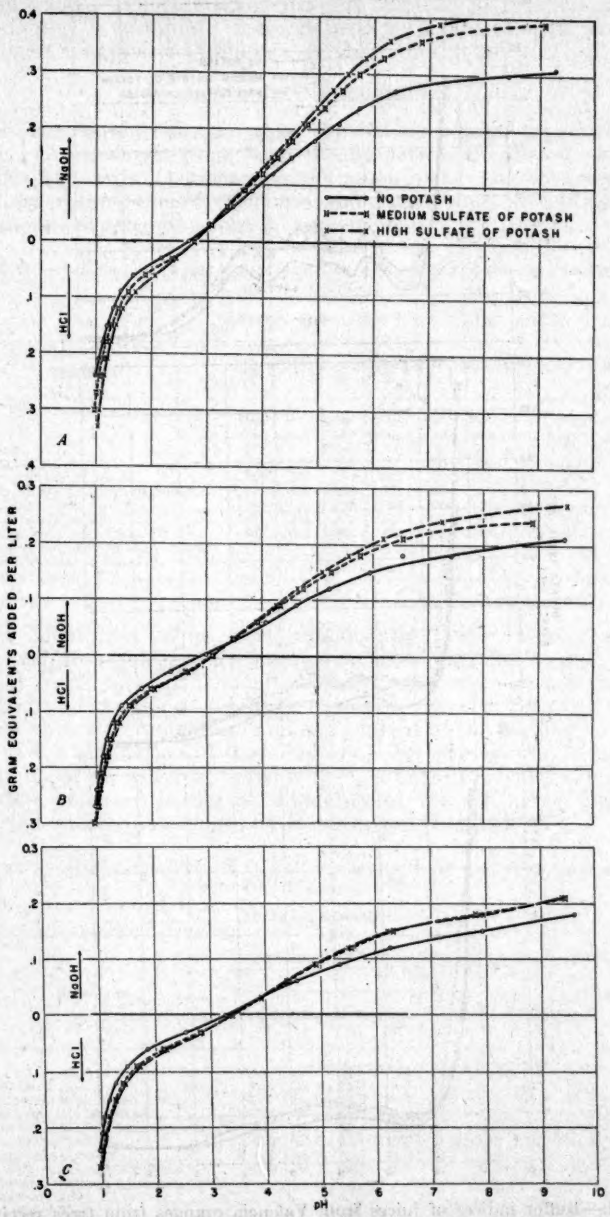

FIGURE 5.—Buffer curves for the juices of Valencia oranges from trees receiving different amounts of sulfate of potash in the fertilizer; *A*, picked September 16, 1939; *B*, picked December 16, 1939; *C*, picked March 8, 1940.

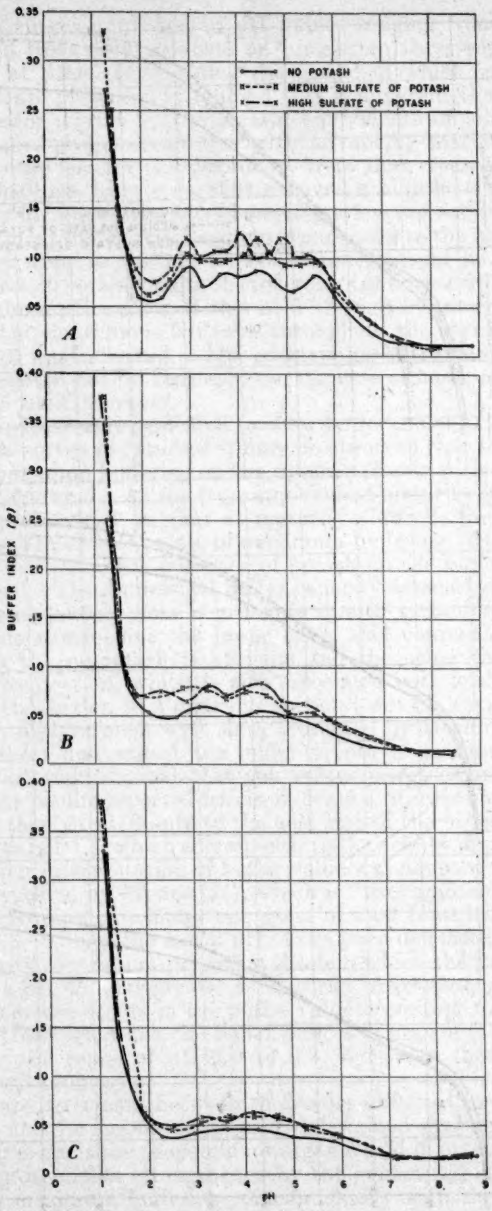

FIGURE 6.—Buffer indices of juices from Valencia oranges from trees receiving different amounts of sulfate of potash in the fertilizer: A, Picked September 16, 1939; *B*, picked December 16, 1939; *C*, picked March 8, 1940.

only in traces. If such acids do occur in the juice of Valencia oranges, the quantity is so minute in comparison with that of citric acid that they exert no apparent influence on the buffer activity of the juice.

ASH AND ITS ALKALINITY

The ash content of the juices showed no regular seasonal trend (table 6). However, juice from the no-potash treatment contained significantly lower (1-percent level) amounts of ash than did juice from any of the potash treatments, and during the period of sampling the amount of ash was about 30 percent lower.

TABLE 6.—*Ash in Valencia orange juice at various dates during the maturation period, 1939–40*

[Means of plot replicates]

Treatment	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
No potash	0.221	0.174	0.183	0.160	0.187	0.203	0.241	0.214	0.229	0.201
Medium sulfate of potash	.381	.269	.289	.296	.261	.291	.331	.286	.347	.306
Medium muriate of potash	.364	.266	.283	.325	.278	.311	.388	.328	.343	.321
Medium sulfate of potash-magnesia	.3.9	.249	.293	.302	.256	.285	.314	.293	.337	.293
High sulfate of potash	.384	.257	.355	.339	.320	.326	.391	.319	.387	.342
High muriate of potash	.331	.265	.301	.356	.300	.288	.371	.291	.334	.315
High sulfate of potash-magnesia	.362	.264	.334	.339	.271	.323	.398	.325	.367	.331
Difference required for significance:										
5-percent level									.060	.023
1-percent level									.091	.031

The alkalinity values were significantly lower (1-percent level) in the ash from juices of fruit grown on trees receiving no potash than in that from juices of fruit grown on potash-treated trees (table 7). Alkalinity of the ash, as might be expected, varies in the same manner as the values for potassium and ash content of the various juice samples and is a function of these factors. No correlations in ash or in alkalinity of the ash were noted with ripening of the fruit. In general, the data reported herein on alkalinity of the ash agree with those reported on Florida oranges by Roberts and Gaddum (*41*).

TABLE 7.—*Alkalinity of the ash of Valencia orange juice at various dates during the maturation period, 1939–40*

[Means of plot replicates]

Treatment	Amount of 0.1 N hydrochloric acid required to neutralize the ash from a 100-gm. sample									
	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
	Ml.	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*
No potash	24.4	25.6	25.3	19.4	24.3	23.7	29.3	24.6	25.9	24.72
Medium sulfate of potash	43.5	39.1	41.9	38.4	44.0	41.4	44.3	38.9	42.3	41.53
Medium muriate of potash	41.8	39.3	42.4	39.9	42.9	43.6	45.6	43.1	42.0	42.28
Medium sulfate of potash-magnesia	38.5	40.9	40.8	38.0	40.5	41.3	42.0	40.0	39.6	40.17
High sulfate of potash	43.2	42.5	43.9	40.3	46.5	43.4	48.5	41.3	46.3	43.98
High muriate of potash	40.7	35.3	40.7	42.0	43.5	40.4	48.8	42.1	39.3	41.42
High sulfate of potash-magnesia	41.6	38.8	44.5	42.9	44.9	46.4	47.8	41.5	44.6	43.67
Difference required for significance:										
5-percent level									4.41	3.01
1-percent level									6.70	4.19

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POTASSIUM

The amount of potassium in the juices fluctuated seasonally in a manner similar to that of the ash. Juice from the no-potash treatment contained about 60 percent of the amount of potassium found in juices derived from potash treatments. This difference is highly significant (table 8).

TABLE 8.—*Potassium content of Valencia orange juice at various dates during the maturation period, 1939–40*

[Means of plot replicates]

Treatment	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
No potash	0.103	0.079	0.079	0.072	0.087	0.092	0.102	0.087	0.102	0.089
Medium sulfate of potash	.148	.119	.128	.132	.135	.150	.158	.137	.166	.141
Medium muriate of potash	.160	.144	.126	.137	.133	.152	.163	.152	.151	.146
Medium sulfate of potash-magnesia	.111	.125	.139	.128	.129	.141	.152	.141	.151	.135
High sulfate of potash	.175	.136	.141	.134	.147	.162	.169	.152	.165	.153
High muriate of potash	.145	.115	.131	.144	.143	.148	.159	.137	.154	.142
High sulfate of potash-magnesia	.180	.125	.138	.136	.132	.161	.168	.137	.165	.149
Difference required for significance:										
5-percent level	------	------	------	------	------	------	------	------	.0171	.0106
1-percent level	------	------	------	------	------	------	------	------	.0260	.0148

From the data presented in tables 6 and 8, it can be readily observed that potassium constitutes a greater portion of the ash of orange juice than do any of the other metals. The salts of citric acid present in the unaltered juice are oxidized to the corresponding carbonates, at the low temperatures (400° to 500° C.) employed in ashing the samples. Thus, it is evident that potassium carbonate constitutes from 70 to 80 percent of the weight of the ash.

The data in tables 6 and 8 have been employed in calculating the percentages of potassium in the ash. These percentages, presented in table 9, show no significant differences between the seasonal-mean potassium content of the ash from juice from the no-potash trees and that from the potash-fed ones. There was, however, a large percentage difference with respect to both the ash and potassium between juices from the no-potash and from the potash-fed trees. Under the conditions imposed in this study with regard to soil and soil amendments, it is apparent that the potassium content of the juice is proportional to the content of the total of the other inorganic constituents of the juice. This finding is entirely in keeping with data reported by other workers (*38*, *41*), who found the potassium content of the ash of orange juice to be 42 to 45 percent, but it is in direct contrast to the data from the inorganic analyses of the leaves taken from the same trees. In the latter case a distinct antagonism was apparent between the amounts of potassium and calcium in the leaf. In leaves in which the potassium content was low the calcium was found to be much higher than normal. The analyses of leaf and juice indicate that the fluctuation in mineral composition, as affected by available soil nutrients, is reflected to a great extent in the leaf, which acts as a regulating mechanism or buffer and serves to maintain a fair degree of constancy in the inorganic constitution of the juice.

TABLE 9.—*Potassium content of the ash of Valencia orange juice at various dates during the maturation period, 1939-40*

[Means of plot replicates]

Treatment	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
No potash	46.6	45.4	43.2	45.0	46.5	45.3	42.3	40.7	44.5	44.4
Medium sulfate of potash	38.9	44.2	44.3	44.6	57.5	51.5	47.7	47.9	47.8	47.2
Medium muriate of potash	44.0	54.1	44.5	42.2	47.8	48.9	42.0	46.3	44.0	46.0
Medium sulfate of potash-magnesia	31.8	50.2	47.4	42.4	50.4	49.5	48.4	48.1	44.8	45.9
High sulfate of potash	45.6	52.9	39 7	39.5	45.9	49.7	43.2	47.6	42.6	45.2
High muriate of potash	43.8	43.4	43.5	40.5	47.7	51.4	42.9	47.1	46.1	45.2
High sulfate of potash-magnesia	49.7	47.3	41.3	40.1	48.7	49.8	42.2	42.2	45.0	45.1
Difference required for significance:										
5-percent level										3.51
1-percent level										4.89

RATIO OF REDUCING TO NONREDUCING SUGARS

The ratios of reducing to nonreducing sugars (table 10; fig. 7), showed a uniform decrease with advancing maturity until January,

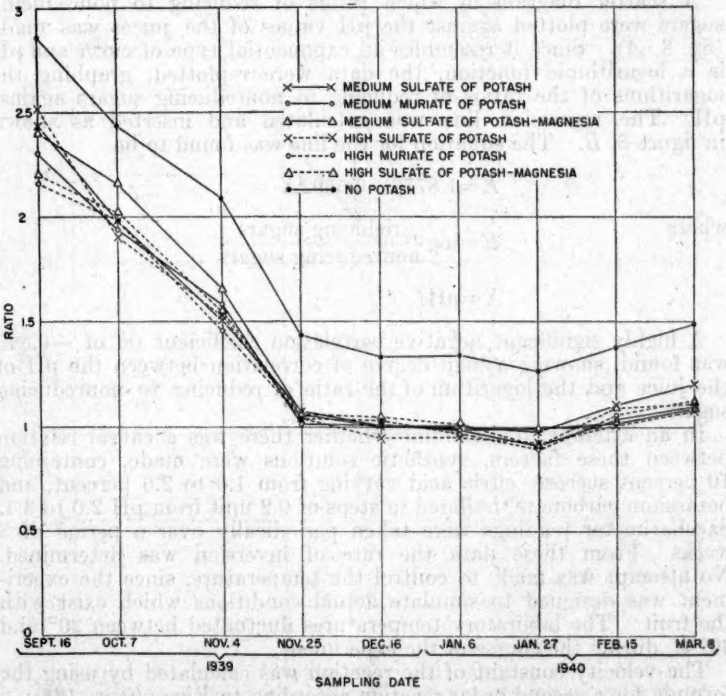

FIGURE 7.—Seasonal changes in the ratios of reducing to nonreducing sugars in the juices from Valencia oranges grown on plots with different amounts of potash and with potash derived from different sources, 1939–40.

when a minimum was reached; after that the ratios increased slightly. The juice from fruit from the no-potash plots contained a significantly

higher ratio of reducing to nonreducing sugars than the juice from fruit from any of the other treatments on each sampling date, although the trends were in the same direction. As the ratios decreased with maturity of the fruit, the pH values of the juice increased.

TABLE 10.—*Ratio of reducing to nonreducing sugars in Valencia orange juice at various dates during the maturation period, 1939–40*

[Means of plot replicates]

Treatment	Sept. 16	Oct. 7	Nov. 4	Nov. 25	Dec. 16	Jan. 6	Jan. 27	Feb. 15	Mar. 8	Mean
No potash	2.86	2.46	2.08	1.43	1.33	1.34	1.26	1.38	1.49	1.74
Medium sulfate of potash	2.43	1.98	1.51	1.07	1.01	1.00	.98	1.11	1.20	1.37
Medium muriate of potash	2.30	1.94	1.58	1.01	1.01	.98	.91	1.03	1.10	1.32
Medium sulfate of potash-magnesia	2.41	2.16	1.65	1.06	1.01	.99	.99	1.02	1.09	1.38
High sulfate of potash	2.52	1.90	1.53	1.04	.95	.95	.92	1.01	1.08	1.32
High muriate of potash	2.16	1.98	1.46	1.04	1.03	.97	.90	1.06	1.12	1.30
High sulfate of potash-magnesia	2.20	2.02	1.54	1.06	1.04	1.01	.91	1.09	1.11	1.33
Difference required for significance:										
5-percent level									.186	.083
1-percent level									.283	.116

A scatter diagram in which ratios of reducing to nonreducing sugars were plotted against the pH values of the juices was made (fig. 8, *A*). Since it resembles an exponential type of curve and pH is a logarithmic function, the data were replotted, graphing the logarithms of the ratios of reducing to nonreducing sugars against pH. The regression line was calculated and inserted as shown in figure 8, *B*. The equation for the line was found to be

$$E=1.8778-0.5692X$$

where

$$E=\log \frac{\text{reducing sugars}}{\text{nonreducing sugars}}$$

$$X=\text{pH}.$$

A highly significant negative correlation coefficient (r) of -0.845 was found, showing a high degree of correlation between the pH of the juice and the logarithm of the ratio of reducing to nonreducing sugars.

In an attempt to determine whether there was a causal relation between these factors, synthetic solutions were made, containing 10 percent sucrose, citric acid varying from 1.0 to 2.6 percent, and potassium carbonate, buffered in steps of 0.2 unit from pH 2.0 to 3.4. Saccharimeter readings were taken periodically over a period of 3 weeks. From these data the rate of inversion was determined. No attempt was made to control the temperature, since the experiment was designed to simulate actual conditions which exist with the fruit. The laboratory temperatures fluctuated between 20° and 30° C. during the course of the experiment.

The velocity constant of the reaction was calculated by using the formula for a second-order reaction according to Rice (*40, p. 123*):

$$k=\frac{1}{at} ln \frac{b}{b-x}$$ [7]

[7] Scatchard (*46, 47*) states that the characteristics of the reaction more nearly approximate those of a sixth-order reaction.

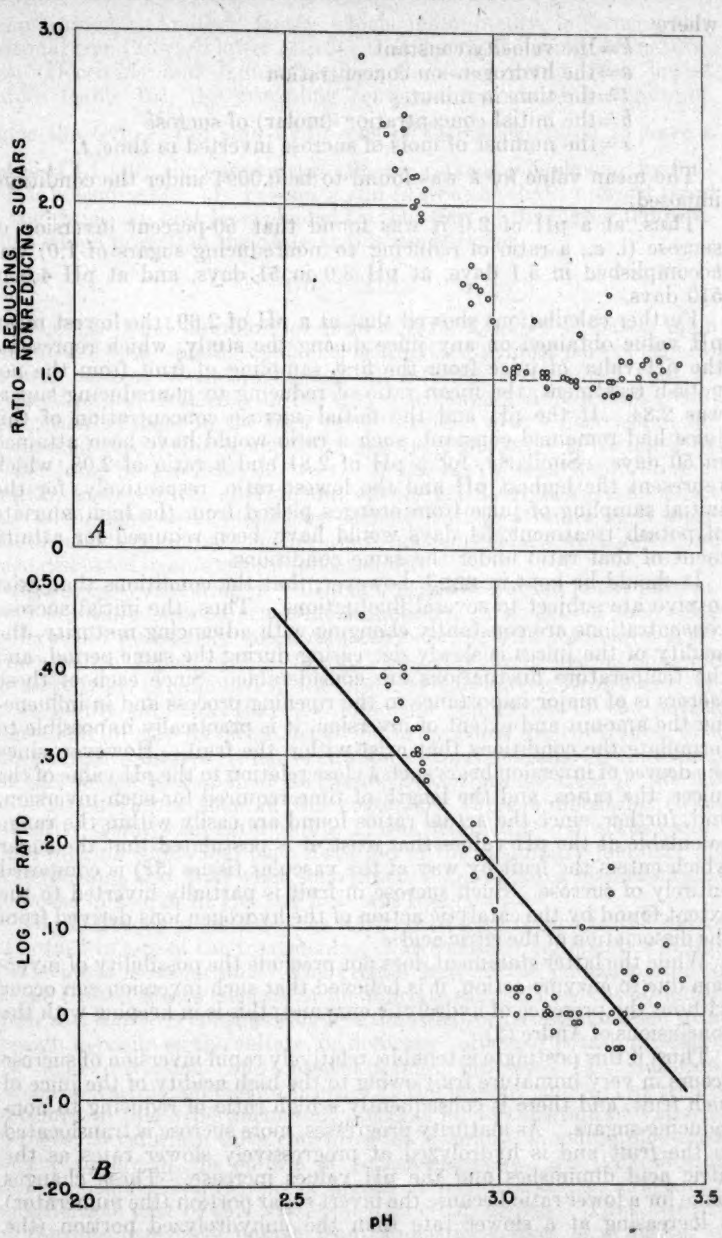

FIGURE 8.—Relation between the hydrogen-ion concentration (pH) of Valencia orange juice and the ratio of reducing to nonreducing sugars, 1939–40: *A*, Scatter diagram; *B*, regression line of pH vs. logarithm of ratio.

where

k=the velocity constant
a=the hydrogen-ion concentration
t=the time in minutes
b=the initial concentration (molar) of sucrose
x=the number of mols of sucrose inverted in time, t.

The mean value for k was found to be 0.0094 under the conditions imposed.

Thus, at a pH of 2.0 it was found that 50-percent inversion of sucrose (i. e., a ratio of reducing to nonreducing sugars of 1.0) was accomplished in 5.1 days, at pH 3.0 in 51 days, and at pH 4.0 in 510 days.

Further calculations showed that at a pH of 2.69, the lowest mean pH value obtained on any juice during the study, which represents the pH value of juice from the first sampling of fruit from the no-potash treatment, the mean ratio of reducing to nonreducing sugars was 2.84. If the pH and the initial sucrose concentration of this juice had remained constant, such a ratio would have been attained in 50 days. Similarly, for a pH of 2.81 and a ratio of 2.08, which represent the highest pH and the lowest ratio, respectively, for the initial sampling of juice from oranges picked from the high muriate of potash treatment, 54 days would have been required for attainment of that ratio under the same conditions.

It should be kept in mind, however, that the conditions that exist in vivo are subject to several fluctuations. Thus, the initial sucrose concentrations are constantly changing with advancing maturity, the acidity of the juices is slowly decreasing during the same period, and the temperature fluctuations are considerable. Since each of these factors is of major importance in the ripening process and in influencing the amount and extent of inversion, it is practically impossible to formulate the conditions that exist within the fruit. However, since the degree of inversion bears such a close relation to the pH value of the juices, the ratios, and the length of time required for such inversion, and, further, since the actual ratios found are easily within the range calculable at the pH values that exist, it is postulated that the sugar which enters the fruit by way of the vascular tissue (*32*) is composed entirely of sucrose. Such sucrose in fruit is partially inverted to the extent found by the catalytic action of the hydrogen ions derived from the dissociation of the citric acid.

While the latter statement does not preclude the possibility of inversion due to enzyme action, it is believed that such inversion can occur without the presence of hydrolytic enzymes; this is in keeping with the conclusions of Andre (*3*).

Thus, if this postulate is tenable, relatively rapid inversion of sucrose occurs in very immature fruit owing to the high acidity of the juice of such fruit, and there is consequently a high ratio of reducing to nonreducing sugars. As maturity progresses, more sucrose is translocated to the fruit and is hydrolyzed at progressively slower rates as the citric acid diminishes and the pH values increase. These changes make for a lower ratio because the invert sugar portion (the numerator) is increasing at a slower rate than the unhydrolyzed portion (the

denominator). Another factor which undoubtedly influences this seasonal trend toward lower ratios is that during the period of maturation (December and January), when the ratios attain their lowest values (table 10), the prevailing temperatures are at a minimum. Since the $Q^{10}\left(\frac{k_{35}}{k_{25}}\right)$ of acid hydrolysis of sucrose is stated to have a value of 4.1 (*40*), the temperature effect in retarding or slowing hydrolysis is considerable. In February and March the slight upward trend in the ratios can best be explained by the effect of the higher temperatures which prevail in the State at this time of year.

DISCUSSION

Since the legal standard of maturity for oranges is based upon the ratio of total soluble solids (consisting for the most part of sugars) to acid content of the juice, and since it has been shown that an induced potash deficiency in the tree is accompanied by a higher concentration of sugar in the fruit juice, together with a decrease in the citric acid content, it is apparent that legal maturity is hastened by withholding potash from the trees. In the case of total deprivation of potash, however, the detrimental effects produced more than offset any advantages to be gained by causing fruit to attain a legal total-soluble-solids-acid ratio sooner than normal. These relations have been discussed in an earlier publication (*6*).

Again, early maturing of Valencia oranges in Florida is not particularly desirable from an economic standpoint. The sale of such early fruit would necessarily enter into competition with midseason fruit. The practical aspect of these findings probably lies in their application to early fruit, exemplified in the State by the Parson Brown variety. It is highly probable that, by judicious application of the potash ingredient of the fertilizer, legal maturity could be controlled to a large extent. Indeed, it has been shown experimentally (*6*) that when all the potash was applied to Parson Brown orange trees in the fall application rather than in the customary three applications, legal maturity (solids-acid ratio of 8 to 1) was attained 2 to 3 weeks earlier than in fruit which had been fertilized in the customary manner. It is significant, also, that when such practice was adopted no measurable decrease in size or in yield of fruit was observed.

No differences of consequence were noted with regard to the source of potash in any of the treatments. The economic significance of this observation is important. The greater part of the potash produced in the United States consists of the muriate salts. The cost of potash from this source is considerably less than that of potash obtained from foreign deposits as the sulfate, or domestic sulfate of potash produced by acid-conversion and base-exchange processes. In addition, foreign potash is difficult to procure at the present time. The results obtained in this experiment indicate that for fertilization of Valencia oranges, the muriate salts constitute as desirable a source of potash as the sulfate or the sulfate of potash-magnesia. In 1937 Florida alone consumed 35,813 tons of potash as fertilizer (*15, p. 401*) and a considerable part of this was applied to citrus groves. Thus, the use of the less expensive potash from domestic sources is a matter of practical importance.

Another point brought out by this experiment is the fact that the critical level of potash fertilization for citrus lies at or below the medium level of application described in these studies; i. e., an annual application of 1.7 to 2.3 pounds of potash. This deduction is readily apparent from the observation that yield and quality of the fruit, as well as some of the biochemical characteristics of the juice, were not materially improved by the application of a fertilizer of higher potassium content. The few benefits obtained were hardly of sufficient value to warrant the additional cost of application of more than the amount of potash contained in the medium mix.

The influence of the potassium content on the hydrogen-ion concentration of the juice is very marked. Ordinarily, if the pH values of two orange juices are compared, the juice that contains the larger quantity of citric acid will also be found to contain the greater concentration of hydrogen ions.

An examination of the data presented herein pertaining to the citric acid content and the pH values of the various juices shows that this general relation does not hold, but that the pH value of the juice is a function of two factors, depending on the citric acid concentration and the ash. It is apparent that on any sampling date juice from the no-potash plots contained considerably less potassium and less citric acid and yet its mean pH value was lower by about 0.10 pH unit than that of juice from any of the potash treatments. This difference becomes more apparent when it is remembered that a difference of 0.10 pH unit means approximately a 20-percent difference in hydrogen-ion concentration.

The increase in the pH value of juice which contains an adequate amount of potassium over that which is deficient is directly attributable to the potassium content of the juice; the differences undoubtedly would be more accentuated were the citric acid content of the juices equal. Thus, juice which contains an adequate amount of potassium can be expected to have a higher pH value than potassium-deficient juice of the same citric acid content.

SUMMARY

Biochemical studies were made of the juice from Valencia oranges taken from trees that received no potash and from trees that received applications of 1.7 to 2.3 and from 2.7 to 3.6 pounds annually of potash per tree from three sources, namely, muriate of potash, sulfate of potash, and sulfate of potash-magnesia.

Potassium deficiency induced by withholding potash for 5 to 5½ years was accompanied by the following changes in the composition of the fruit juice, when compared with fruit juice from trees supplied with potash in the fertilizer: (1) An increased concentration of reducing sugars; (2) an increased concentration of total sugars; (3) a decreased concentration of ascorbic acid; (4) a decreased concentration of citric acid; (5) a decrease in the buffer index of the juice; (6) a decrease in the pH value of the juice; (7) a decrease in the percentages of ash and potassium and in the alkalinity of the ash; (8) an increase in the ratio of reducing to nonreducing sugars.

Inasmuch as legal maturity is based on the ratio of total soluble solids to acid in the juice, potassium deficiency induces a 2- to 3- week earlier onset of legal maturity.

The belief commonly held that muriate of potash in the fertilizer exerts a harmful effect on the quality of Valencia oranges is not substantiated by the results of these experiments. No differences attributable to the source of potash were found in the juice of oranges from plots that received muriate of potash, sulfate of potash, or sulfate of potash-magnesia.

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INHERITANCE OF ASCORBIC ACID CONTENT IN SNAP BEANS[1]

By B. L. WADE, *principal geneticist*, P. H. HEINZE, *associate plant physiologist*, MARGARET S. KANAPAUX, *junior physiologist*, and CHARLOTTE F. GAETJENS, *junior scientific aide, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

In a program of breeding hardy, productive snap beans (*Phaseolus vulgaris* L.) adapted for growing in the southeastern United States, it was found that unless some attention were given to ascorbic acid (vitamin C) content the hardy strains developed were likely to have less ascorbic acid than varieties commonly grown in the Southeast, such as Stringless Black Valentine and Bountiful.[2] The decrease in ascorbic acid content was probably due to the fact that the hardy parents, U. S. No. 5 Refugee and closely related lines, have an average ascorbic acid content lower than that of the commonly grown varieties. Some crosses appeared to be segregating widely, so a genetic study was undertaken in the hope that a basis might be found for solving the breeding difficulty. The investigation was made in 1942 at the United States Regional Vegetable Breeding Laboratory, Charleston, S. C.

MATERIALS AND METHODS

Notes were kept on broken and diseased plants, but data obtained from these plants were ordinarily not used in compiling the results. The inclusion of data from broken and diseased plants increases the variability, since when injury first occurs the ascorbic acid content is usually high but with the death or senescence of the plant ascorbic acid may be very low. Where a small amount of some disease was noted it was indicated as being present, but the data were not discarded. Little breaking or disease was observed.

The cross used in this study was U. S. No. 5 Refugee × Blue Lake and its reciprocal. Preliminary studies of F_2's of this cross were made in the spring of 1942. In the summer 4 F_3 families derived from F_2 plants having very high or very low ascorbic acid content were grown. In the fall a reserve lot of F_2 material, 25 F_3 families, and the same number of the reciprocal were studied. Two substrains of each of the parent varieties, as well as 2 F_4 families of the cross and 1 of the

[1] Received for publication July 22, 1943. This work was performed under an allotment from the Special Fund authorized by Title I of the Bankhead-Jones Act of June 29, 1935.

[2] WADE, B. L., and KANAPAUX, M. S. ASCORBIC ACID CONTENT OF STRAINS OF SNAP BEANS. Jour. Agr. Res. 66: 313-324, illus. 1943.

reciprocal, were also studied in the fall. Single-plant selections of the snap varieties were used in the cross and the same ones as controls when the F_2 and F_3 progenies were being observed.

Approximately 75 seeds were planted in each 32-foot row, and samples were taken as soon as the pods were considered to have arrived at an edible stage. Pods of U. S. No. 5 Refugee and Blue Lake were harvested as checks each day that pods of the hybrids were harvested. Harvesting usually began at 7:30 a. m. and was complete by 11 a. m. Ascorbic acid determinations were made immediately or else on material held not longer than 24 hours with high humidity at a temperature of 36° F. Records of all plants were kept on an individual basis, and an effort was made to obtain samples from each plant at two different periods, preferably about 1 week apart. A study of the influence of size and position of pods on the plant on the ascorbic acid content of the U. S. No. 5 Refugee parent was also made.

Approximately 2,300 ascorbic acid determinations were made in this work in 1942, of which about 500 made on the spring crop were considered as only exploratory in nature.

A modification of the Morell [3] method was used for the determination of ascorbic acid. From 25 to 40 gm. of the bean pods was extracted with 200 ml. of 1-percent metaphosphoric acid for 2 minutes in a Waring blender. The extract was filtered through a fluted Whatman No. 12 filter paper, and the first portion of the filtrate was discarded. A 1–ml. aliquot was placed in a colorimeter tube containing 10 ml. of a solution of 2,6-dichlorophenolindophenol (concentration, 17 mg. per liter) and read in an Evelyn photoelectric colorimeter within 30 seconds. Satisfactory readings were obtained when the concentration of the extract was between 20 and 70 micrograms of ascorbic acid per milliliter. Moisture determinations were made, and the dilution effect of the moisture in the sample was considered in calculating the ascorbic acid.

The procedure just described eliminates the use of buffers without loss of accuracy and increases the number of determinations that can be made per day.

RESULTS

In the spring of 1942, the F_2 progenies from U. S. No. 5 × Blue Lake gave a mean ascorbic acid content of 23.84 mg. per 100 gm. (fresh weight), with a standard deviation of 5.54, and those from the reciprocal cross gave 22.45 ± 5.69 mg. The ranges were 11.2 to 28.5 and 10.9 to 33.4 mg., respectively. Owing to an accident, the strains used as parents did not mature at the same time as the F_2 so that other U. S. No. 5 Refugee and Blue Lake strains were used as substitutes for the parents. These gave 22.01 ± 3.42 and 18.94 ± 2.47 mg., respectively.

In the summer of 1942, as previously stated, a small planting of parents and F_3 seed from the high and low lines of the spring-grown F_2 of the U. S. No. 5 Refugee × Blue Lake and reciprocal was made. The U. S. No. 5 Refugee parent averaged 19.5 mg. per 100 gm. in ascorbic acid and the Blue Lake parent 15.1 mg. per 100 gm. The averages for the high and low lines of the cross and its reciprocal were 11.0

[3] MORELL, S. A. RAPID PHOTOMETRIC DETERMINATION OF ASCORBIC ACID IN PLANT MATERIALS. Indus. and Engin. Chem., Analyt. Ed. 13: 793–794, illus. 1941.

and 31.7 mg. and 11.8 and 32.8 mg., corresponding to spring values of 11.2 and 28.5 mg. and 10.9 and 33.4. mg., respectively. The requirement for significant difference at the 5-percent level was 2.2 for parents and hybrids when combined in a variance analysis.

In the fall of 1942 the mean ascorbic acid value for the F_2 of U. S. No. 5 Refugee × Blue Lake was 19.5 mg. per 100 gm. with a standard deviation of 4.30, the range of values being from 12.2 to 32.4 mg.; for the reciprocal the mean value was 16.0±3.08 mg., with a range of 10.5 to 22.6 mg. These values are fairly close together, so the data were combined for presention in figure 1, *C*.

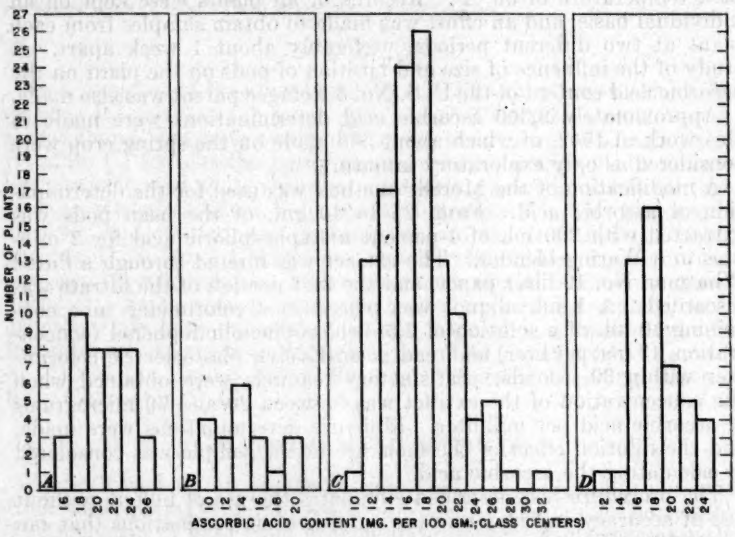

FIGURE 1.—Distribution with respect to ascorbic acid content of (*A*) U. S. No 5 Refugee plants, (*B*) Blue Lake plants, (*C*) F_2 plants of U. S. No. 5 Refugee × Blue Lake and the reciprocal, and (*D*) F_3 families of the same crosses. Mean ascorbic acid content: *A*, 20.9±2.97; *B*, 14.2±3.20; *C*, 17.7±4.03; *D*, 17.5±4.39.

The F_3 of the cross U. S. No. 5 Refugee × Blue Lake had a mean ascorbic acid content of 17.9 mg. per 100 gm., with a standard deviation of 4.25, and the F_3 of the reciprocal cross had a value of 17.1±4.49 mg. The range was from 7.6 to 31.6 mg. for the cross, and from 6.0 to 35.0 mg. for the reciprocal based on single plants. The range of F_3 family means was 13.3 to 23.7 for the cross and 13.7 to 22.8 for the reciprocal. Since the values for this cross and its reciprocal are very close, they were combined to give the results shown in figure 1, *D*.

One F_4 family from the cross showed a mean of 12.0 mg. per 100 gm. and one from the reciprocal showed 11.8, while another family from the cross averaged 30.0 mg.

Two substrains of the U. S. No. 5 Refugee gave ranges of 16.7 to 26.8 mg. per 100 gm. and 16.4 to 25.2 mg., with means of 20.9 and 20.9 mg. and standard deviations of 2.77 and 2.89, respectively. Two substrains of Blue Lake gave ranges of 9.2 to 16.7 mg. and 11.9 and 19.8

mg., with means of 11.8 and 15.3 mg. and standard deviations of 2.73 and 3.04, respectively. For each variety the two substrains were combined in a single distribution in figure 1, *A* and *B*.

At one time during the harvesting season a study was made to determine whether the size of pods and their position on the plant influenced the ascorbic acid content. This study was confined to the U. S. No. 5 Refugee parent. Single plants were divided into an upper and lower part and the pods from each part were divided into medium and large. The medium pods averaged 17.5 mg. of ascorbic acid per 100 gm., and the large pods 20.5 mg.; the upper pods averaged 18.7 mg. and the lower pods 19.4 mg. The requirement for significant difference is 1.3. The average for medium pods was the same whether from the upper or the lower part, but large pods from the lower part averaged 21.2 mg. as compared with 19.8 for large pods from the upper part, with 1.9 required for significant difference.

DISCUSSION

The means for ascorbic acid content of the F_2 and the F_3 populations of the fall crop were very close to the mean of the U. S. No. 5 Refugee and Blue Lake parents. The variance (standard deviation squared) for the parents was approximately half the variance for the segregating generations (9.38 vs. 16.21 for F_2 and 19.25 for F_3). From these values it might have been possible to calculate the number of genes involved, except that there is evidence of transgressive segregation. Both F_2 and F_4 gave ascorbic acid values beyond the upper range of the parents. Reynard and Kanapaux,[4] in a preliminary genetic study of ascorbic acid content of tomatoes, found the content of hybrids intermediate between those of the two parents.

Because of unfavorable weather conditions, snap beans are ordinarily not grown in the Charleston, S. C., area during the summer months, but owing to the possible breeding value of some of the lines high in ascorbic acid content, a small summer planting was made of parents and of high and low F_3 lines of the cross U. S. No. 5 Refugee × Blue Lake and of the reciprocal cross. These F_3 lines gave means near the values obtained for the corresponding F_2 in the spring. Reduction in variance is probably due to uniformity of harvest (all samples in 1 day) and to an approach to genetic uniformity for high and low ascorbic acid values in the F_3 lines.

The study of influence of position and size of pods on ascorbic acid content gave an error variance of 5.02, indicating that the remainder of the U. S. No. 5 Refuge variance for the fall of 1942 (7.84−5.02=2.82) was due to the influence of time of picking and other factors within the picking season. Since these other factors cannot be readily controlled it is necessary to increase the number of observations.

[4] REYNARD, G. B., and KANAPAUX, M. S. ASCORBIC ACID (VITAMIN C) CONTENT OF SOME TOMATO VARIETIES AND SPECIES. Amer. Soc. Hort. Sci. Proc. 41: 298-300. 1942.

SUMMARY AND CONCLUSIONS

In a genetic study of snap beans involving considerable preliminary work it was found that in the cross U. S. No. 5 Refugee × Blue Lake and its reciprocal the quantity of ascorbic acid is a heritable character and that there is evidence of transgressive segregation. Although the spring-grown F_2 is considered as giving only preliminary information it indicated transgression, and this indication was confirmed by a summer-grown crop of F_3 lines selected for high and low ascorbic acid content. These high and low F_3 lines were all significantly different at the 1-percent level from the parent lines and close to the values observed for the corresponding F_2 plants.

In the fall the F_2 population again indicated transgressive segregation, but the F_3 means did not. The 50 F_3 families taken strictly at random from the F_2 plants did not include any families with average values significantly above or below the parental values. However, some F_3 families had as wide a range as the F_2. Some additional evidence of transgression was found in 3 F_4 families.

It was found that much of the variability in ascorbic acid content of pods of U. S. No. 5 Refugee was due to size and position on the plant, i. e., a variance of 5.02 for size and position with a remaining variance of 2.82 for all other factors involved during the fall of 1942.

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